Risks from *Ebolavirus* Discharge from Hospitals to Sewer Workers

Charles N. Haas¹, Taylor Rycroft¹*, Kyle Bibby², Leonard Casson²

**ABSTRACT:** Current World Health Organization and Centers for Disease Control and Prevention guidance for the disposal of liquid waste from patients undergoing treatment for *Ebolavirus* disease at hospitals in the U.S. is to manage patient excreta as ordinary wastewater without pretreatment. The potential for *Ebolavirus* transmission via liquid waste discharged into the wastewater environment is currently unknown, however. Possible worker inhalation exposure to *Ebolavirus*-contaminated aerosols in the sewer continues to be a concern within the wastewater treatment community. In this study, a quantitative microbial risk assessment was carried out to assess a sewer worker’s potential risk of developing *Ebolavirus* virus disease from inhalation exposure when performing standard occupational activities in a sewer line serving a hospital receiving *Ebolavirus* patients where there is no pretreatment of the waste prior to discharge. Risk projections were estimated for four scenarios that considered the infectivity of viral particles and the degree of worker compliance with personal equipment guidelines. Under the least-favorable scenario, the median potential risk of developing *Ebolavirus* virus disease from inhalation exposure to *Ebolavirus*-contaminated aerosols in the sewer is approximately $10^{-5.77}$ (with a first to third quartile range of $10^{-7.06}$ to $10^{-4.65}$), a value higher than many risk managers may be willing to accept. Although further data gathering efforts are necessary to improve the precision of the risk projections presented here, the results suggest that the potential risk that sewer workers face when operating in a wastewater collection system downstream from a hospital receiving *Ebolavirus* patients warrants further attention, and that current authoritative guidance for *Ebolavirus* liquid waste disposal—to dispose in the sanitary sewer without further treatment—may be insufficiently protective of sewer worker safety. *Water Environ. Res.*, **89**, 354 (2017).

**KEYWORDS:** *Ebolavirus*, risk assessment, wastewater, aerosol, inhalation, sewer worker.

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**Introduction**

In March 2014, the first cases of the 2014–16 *Ebolavirus* outbreak were officially reported in Guinea. As of April 13, 2016, the number of documented cases and deaths had risen to 28,616 and 11,310, respectively, across Guinea, Liberia, and Sierra Leone, making the outbreak the largest and most damaging since the virus was discovered in 1976 (World Health Organization [WHO], 2015; Centers for Disease Control and Prevention [CDC], 2016). The *Ebolavirus* genus is classified as a member of the virus family Filoviridae (Piercy et al., 2010). To date, five species of *Ebolavirus* (EBOV) have been identified: Zaire, Sudan, Bundibugyo, Reston, and Tai Forest, all of which are enveloped, single-stranded, negative-sense RNA viruses (Beer et al., 1999).

The virus responsible for the 2014–16 outbreak in West Africa belongs to the *Zaire* species. *Ebolavirus* Zaire causes an acute, serious illness in humans known as *Ebolavirus* disease (EVD), with typical symptoms of fever, vomiting, diarrhea, rash, impaired kidney and liver function, and in some cases both internal and external bleeding. *Ebolavirus* disease is often fatal if untreated (WHO, 2015).

*Ebolavirus* is primarily transmitted from person to person through direct contact with the bodily fluids of infected individuals, such as blood, diarrhea, stool, vomit, urine, sweat, saliva, and tears, with the highest virus concentrations typically found in blood (Johnson et al., 1995; Peters et al., 1996). Transmission via inhalation of aerosolized EBOV particles is also a potential risk, although it is believed to be less likely than direct contact (Judson et al., 2015). Although there is no confirmed epidemiological evidence of aerogenic infection by EBOV in humans, airborne particle transmission could not be ruled out for twelve EVD patients identified during the 1995 EBOV Zaire outbreak in Kikwit, Democratic Republic of the Congo (Roels et al., 1999). In monkeys, successful infection after respiratory administration of EBOV Zaire has been demonstrated in various species (Johnson et al., 1995; Reed et al., 2011). Separately, aerosolized EBOV Zaire was cited as the likely natural transmission route between infected monkeys and healthy experimental controls housed in the same room within a biocontainment laboratory (Jaax et al., 1995).

Other EBOV strains have also been implicated in aerosol transmission to monkeys. The *Sudan* strain has been demonstrated to be aerogenically infectious through mechanical
inhaled as an aerosol. In nonhuman primate studies, the LD\textsubscript{50} for the inhalation transmission pathway has been shown to be less than 10 plaque forming units (PFUs) (Franz et al., 1997; Reed, 2011).

Until August 2014, when two missionaries developed EVD while working at a clinic in Liberia and were evacuated to Emory University Hospital, EVD had never been encountered or treated in humans in the U.S. (Stephens, 2015). Over the course of the 2014–16 outbreak, 11 individuals have been treated at hospitals in the U.S. Managing the solid and liquid waste from EVD patients in the U.S. is a significant challenge. Proper disposal of EBOV-contaminated waste is required to prevent subsequent accidental infections. A single patient can generate more than 450 kg of regulated solid waste throughout treatment (mostly personal protective equipment [PPE]) (Lowe et al., 2014), and up to 10 L of infectious liquid waste per day (mostly watery diarrhea) (Stephens et al., 2015). The potential for EBOV transmission via liquid waste discharged into the wastewater environment is currently unknown. Possible worker inhalation exposure in the sewers and at wastewater treatment facilities continues to be a concern within the wastewater treatment community.

In interim guidance provided in August 2014, WHO suggested that liquid waste from Ebola patients could be safely discharged in the sanitary sewer without disinfection:

"Waste, such as faeces, urine and vomit, and liquid waste from washing, can be disposed of in the sanitary sewer or pit latrine. No further treatment is necessary" (WHO, August 2014).

In December 2014, WHO issued updated, more conservative guidance without explicitly recommending pretreatment of liquid waste prior to disposal:

"Waste, such as faeces, urine and vomit, and liquid waste from washing, can be disposed of in the sanitary sewer or in pit latrines dedicated to HF patients. Standard precautions should be taken to prevent contamination of the environment by faeces and urine. Ebola is likely to inactivate significantly faster in the environment than enteric viruses with known waterborne transmission (e.g., norovirus, hepatitis A virus). Containing excreta for a period of time in a closed tank (at least a week) could allow for natural virus declines. Two tank systems with parallel tanks would help to facilitate this, as one tank could be used until full, then allowed to sit while the next tank is being filled" (WHO, December 2014).

In similar guidance to WHO, the CDC offers the following advice for EBOV disposal:

"Sanitary sewers may be used for the safe disposal of patient waste. Additionally, sewage handling processes in the United States are designed to inactivate infectious agents” (CDC, 2014a).

In contrast to WHO and CDC direction, some experts have warned that all human waste and bodily excretions from a patient with viral hemorrhagic fever (which includes EBOV) should be considered infectious and should be disinfected prior to disposal into a municipal sewer system or septic tank by adding disinfectant prior to use or by using chemical toilets (Bausch and Peters 2009; Peters et al., 1996). Inconsistent guidance, combined with a lack of data surrounding EBOV persistence in unsterilized wastewater and the absence of a risk-based approach for waste handling (Bibby et al., 2015), creates uncertainty as to whether sewer workers are adequately protected from environmental transmission of EBOV via aerosolized wastewater.

This study seeks to assess the potential risks to sewer workers in the sewer line serving a hospital receiving Ebola patients by performing a Quantitative Microbial Risk Assessment (QMRA), including an exposure assessment, dose-response assessment, and probabilistic analysis to determine the magnitude and uncertainty of the resultant risks. Study results and recommendations for areas of future research are presented here.

**Materials and Methods**

To determine the magnitude and uncertainty of the potential risk to collection system workers in the sewer line serving a hospital receiving Ebola patients, a QMRA was performed.

**Quantitative Microbial Risk Assessment Description.** Quantitative Microbial Risk Assessment is an established methodology used to estimate the risk of adverse health consequences resulting from exposure to a pathogen (Haas et al., 2014). It has been applied in the development of standards for wastewater, drinking water, and food safety (Haas, 2015). Similar to chemical risk assessment, the QMRA construct is comprised of 4 elements (Haas, 2014):

1. Hazard identification (identification of the microorganism and the spectrum of human illnesses and disease associated with it),
2. Exposure assessment (determination of the extent of a population’s exposure in terms of number of microorganisms encountered),
3. Dose-response assessment (characterization of the relationship between the number of microorganisms encountered and the probability of a health effect), and
4. Risk characterization (estimation of the magnitude, variability, and uncertainty of the health risk in the exposed population).

As the hazard identification step for *Ebolavirus Zaire* strain is well-documented in the literature, this investigation focused on the latter three steps of the QMRA framework.

**Exposure Assessment.** Inhalation of aerosols containing EBOV viral particles has been documented as a potentially hazardous event (Dalgard et al., 1992; Jaax et al., 1995; Johnson et al., 1995; Roels et al., 1999; Reed et al., 2011). The exposure...
assessments performed in this study estimated the dose of EBOV viral particles a sewer worker inhales per exposure in the sewer.

**Scenario of Interest.** The scenario of interest was defined as a collection system worker operating in a municipal sewer line serving a hospital treating a single Ebola patient where there is no pretreatment of the patient’s liquid waste prior to discharge. The point of worker exposure was assumed to be immediately downstream of the hospital sanitary discharge into the municipal interceptor.

**Input Parameters.** The input parameters required to calculate the number of respirable EBOV viral particles a sewer worker inhales per exposure were defined and are shown in Table 1. A detailed literature search was carried out and values for each parameter were obtained from experimental data in the literature or from consultations with subject matter experts. Consultations with subject matter experts involved phone and e-mail conversations with members of the sanitation departments from two major U.S. metropolitan districts.

All inputs were characterized by point values or distributions, as justified by the available data. Distributions were used when inputs possessed known elements of variability or uncertainty. Distributions were fit to data found in the literature using Oracle Crystal Ball 11.1.2.4.000 Classroom Edition (interfaced with Microsoft Excel 2013) and ranked by the Anderson–Darling goodness of fit statistic. The distribution with the lowest Anderson–Darling goodness of fit statistic was selected as the best fit. Due to software limitations, when fewer than 15 values for an input existed, a comparison of distribution fits could not be made; therefore, a distributional form was assumed and the parameters estimated.

Where information pertinent to EBOV was unavailable, values were inferred based on other pathogens and expert judgment. Values and distributions for the exposure assessment input parameters are summarized in Table 2.

**Statistical Analysis.** Equation 1 was developed to determine the dose of respirable EBOV viral particles inhaled by a sewer worker per exposure, in terms of RNA copies (EBOV, like other microorganisms, is often measured by quantitative PCR). Because EBOV is an RNA virus, it is measured by a reverse transcriptase qPCR and reported in units of genome copies. Not all genome copies may arise from infectious virions, but, in a viability assay (such as plaquing or tissue culture infectious dose), not all infectious particles may be readily quantified.). Parameter values were input into eq 1 as point values or distributions and a Monte Carlo simulation was run with 10,000 trials using Crystal Ball. A sensitivity analysis was performed using Crystal Ball to determine which parameters had the greatest impact on the variability of the exposure results. The 10,000 dose estimates from the Monte Carlo simulation were extracted and used in the risk characterization step of the QMRA.

This process was repeated with a conversion factor applied to eq 1 in order to get the 10,000 dose estimates in terms of PFUs. The conversion factor is discussed in the section titled “Excreta and Secreta Concentrations of EBOV”.

Equation 1, below, is the calculation for number of respirable EBOV RNA copies inhaled and retained in lungs per exposure.

\[
D_{\text{EBOV,pe}} = \frac{Q \times 10^{C_{FS}} \times 1000 \times \frac{D_H}{D_I} \times \frac{1}{10^{\text{FRem}}} \times 10^{P_C}}{\text{FResp} \times I \times (1 - \text{FRem}) \times I_H \times 60} \tag{1}
\]

where,

- \( D_{\text{EBOV,pe}} \) = Number of respirable EBOV RNA copies inhaled and retained in lungs per exposure,
- \( Q \) = daily patient excreta and secreta production to sewer (liters excreta/secrata per day),
- \( C_{FS} \) = excreta and secretas concentrations of EBOV (\( \log_{10} \) viral RNA copies per mL excreta),
- \( D_H \) = hospital daily outflow (liters per day),
- \( D_I \) = interceptor daily flow (liters per day),
- \( R \) = EBOV die-off rate (\( \log_{10} \) reduction per second),
- \( t_T \) = sewer travel time from patient to point of worker exposure (seconds),
- \( P_C \) = partition coefficient (\( \log_{10} \) pathogens per m\(^3\) sewer headspace/pathogens per m\(^3\) wastewater),
- \( F_{\text{Resp}} \) = respirable fraction (unitless),
- \( I \) = worker inhalation rate (liters per minute),
- \( F_{\text{Rem}} \) = EBOV removal fraction by worker ppe (unitless),
- \( I_H \) = time spent by a sewer worker in the proximity (hours).

**Dose-Response Assessment.** In work separate from this project, Haas along with Professors Joan Rose and Jade

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient excreta and secreta production to sewer</td>
<td>Literature</td>
</tr>
<tr>
<td>Excreta and secreta concentrations of EBOV</td>
<td>Literature</td>
</tr>
<tr>
<td>Hospital daily outflow (for internal dilution rate in hospital)</td>
<td>Subject matter experts</td>
</tr>
<tr>
<td>Interceptor daily flow (for dilution from hospital discharge to sewer to point of worker exposure)</td>
<td>Subject matter experts</td>
</tr>
<tr>
<td>EBOV die-off rate</td>
<td>Literature</td>
</tr>
<tr>
<td>Sewer travel time from patient to point of worker exposure</td>
<td>Literature</td>
</tr>
<tr>
<td>Partition coefficient (ratio of aerosol concentration of EBOV to liquid concentration)</td>
<td>Literature</td>
</tr>
<tr>
<td>Respirable fraction (fraction of aerosols generated that are respirable)</td>
<td>Literature</td>
</tr>
<tr>
<td>Worker inhalation rate</td>
<td>Literature</td>
</tr>
<tr>
<td>EBOV removal fraction by worker PPE</td>
<td>Literature</td>
</tr>
<tr>
<td>Time spent by a sewer worker in the proximity</td>
<td>Subject matter experts</td>
</tr>
</tbody>
</table>
Table 2—Values and distributions for inputs to exposure assessment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value/Range</th>
<th>Distribution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient excreta and secreta production to sewer</td>
<td>Liters excreta/secreta per day</td>
<td>2–10</td>
<td>Logistic (Mean = 5.89; Scale = 1.04)</td>
<td>(Bishop et al., 2014; Chertow et al., 2014; Kreuels et al., 2014; Lowe et al., 2014; Lyon et al., 2014; Ribner, 2014; Roberts and Perner 2014; Ker et al., 2015)</td>
</tr>
<tr>
<td>Excreta and secreta concentrations of EBOV</td>
<td>Viral RNA copies per mL excreta</td>
<td>2.8–7.2log_{10}</td>
<td>Logistic (Mean = 4.38log_{10}; Scale = 0.61log_{10})</td>
<td>(Towner et al., 2004; Kreuels et al., 2014; Wolf et al., 2014; Casanova and Weaver, 2015)</td>
</tr>
<tr>
<td>Hospital daily outflow (for internal dilution rate in hospital)</td>
<td>Liters per day</td>
<td>$3.63 	imes 10^5–1.33 	imes 10^6$</td>
<td>Lognormal (Mean = 8.27 × 10^3; Std. Dev. = 4.06 × 10^3)</td>
<td>MWRDGC*</td>
</tr>
<tr>
<td>Interceptor daily flow (for dilution from hospital discharge to sewer to point of worker exposure)</td>
<td>Liters per day</td>
<td>$1.82 	imes 10^7 – 3.29 	imes 10^8$</td>
<td>Lognormal (Mean = 1.61 × 10^6; Std. Dev. = 1.50 × 10^5)</td>
<td>MWRDGC*</td>
</tr>
<tr>
<td>EBOV die-off rate</td>
<td>Log_{10} reduction per second</td>
<td>$6.43 	imes 10^{-6}$</td>
<td>Point</td>
<td>(Piercey et al., 2010; Smither et al., 2011; Bibby et al., 2015; Fischer et al., 2015)</td>
</tr>
<tr>
<td>Sewer travel time from patient to point of worker exposure</td>
<td>Seconds</td>
<td>0 (Negligible compared to die-off rate)</td>
<td>Point</td>
<td>(Weissbrodt et al., 2009; Ort et al., 2010)</td>
</tr>
<tr>
<td>Partition coefficient (Ratio of aerosol concentration of EBOV to liquid concentration)</td>
<td>Pathogens per m^3 sewage headspace / pathogens per m^3 wastewater</td>
<td>$-11.46 – 5.88log_{10}$</td>
<td>Beta (Min = $-11.46log_{10}$; Max = $-5.88log_{10}$; Alpha = $2.3281log_{10}$; Beta = $1.96512log_{10}$)</td>
<td>(Haas et al., 2002; Dutkiewicz 2003; Medema et al., 2004; Karra and Katsivela, 2007; Korzeniewska et al., 2009; Haas et al., 2010; Hung et al., 2010; Stellaeci et al., 2010; Korzeniewska, 2011)</td>
</tr>
<tr>
<td>Respirable fraction (fraction of aerosols generated that are respirable)</td>
<td>Unitless</td>
<td>0.28–1.00</td>
<td>Uniform† (Min = 0.28; Max = 1.00)</td>
<td>(Dutkiewicz, 2003)</td>
</tr>
<tr>
<td>Worker inhalation rate</td>
<td>Liters per minute</td>
<td>$51.0 ± 29.8$</td>
<td>Normal (Mean = 51.0; Std. Dev. = 14.9)</td>
<td>(U.S. EPA, 2011)</td>
</tr>
<tr>
<td>EBOV removal fraction by worker PPE</td>
<td>Respiratory PPE used:</td>
<td>0.95–0.99</td>
<td>Respiratory PPE used:</td>
<td>(Rengasamy et al., 2004; Balazy et al., 2006a; Balazy et al., 2006b; Gupta, 2011; Wen et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>No respiratory PPE used:</td>
<td>0</td>
<td>No respiratory PPE used:</td>
<td></td>
</tr>
<tr>
<td>Time spent by a sewer worker in the proximity</td>
<td>Hours</td>
<td>0.5–4</td>
<td>Uniform† (Min = 0.5; Max = 4.0)</td>
<td>MWRDGC*, LACSD**</td>
</tr>
<tr>
<td>Conversion from EBOV RNA copies to PFUs</td>
<td>EBOV RNA copies per PFU</td>
<td>$3–4log_{10}$</td>
<td>Uniform† (Min = $10^3$; Max = $10^4$)</td>
<td>(Towner et al., 2004)</td>
</tr>
</tbody>
</table>

* MWRDGC: Metropolitan Water Reclamation District of Greater Chicago.
* LACSD: Sanitation Districts of Los Angeles County.
† Insufficient data were available to compare distributions using Crystal Ball. A distribution was therefore assumed and the parameters were estimated based on the data available.

Mitchell (both from Michigan State University) have developed a dose-response relationship for inhaled EBOV using published nonhuman primate (NHP) data (Mitchell, in preparation). The dose metric in these studies were PFU. Based on this analysis, conducted in R version 3.2.1 (The R Foundation for Statistical Computing), the best-fit dose-response model was found to be the exponential dose-response distribution (Haas et al., 2014):

Equation 2, below, is the exponential dose-response model.

$$P_I(d) = 1 - \exp(-k^*d)$$  \hspace{1cm} (2)

where,

$$P_I(d) = \text{probability of infection},$$

$$d = \text{average exposure dose},$$

$$k = \text{dose-response parameter specific to EBOV},$$

$$N_{50} = \text{median effective dose (exposure dose that would result in half the exposed population experiencing the specified health endpoint), where: } N_{50} = \left(\ln \left(0.5\right) \right) / -k$$

The exponential model was selected and a bootstrap sampling distribution was obtained for the dose-response parameter ($k$) by performing 500 bootstrap iterations in R. The set of bootstrapped dose-response parameters was used in the risk characterization step of the QMRA.
Haas et al.

**Risk Characterization.** The risk characterization performed in this study provided a quantitative probabilistic estimate of risk of EVD illness for a typical sewer worker operating in a collection system downstream from a hospital treating a single Ebola patient.

The set of 10,000 replicates returned from the exposure assessment and the set of 500 dose-response parameters \( \beta \) from the dose-response assessment were used as inputs into the exponential dose-response model (eq 2). A Monte Carlo simulation was run with a total of 1000 trials using R Studio Version 0.99.467 (RStudio, Inc.). In each trial, a random exposed dose was selected from the set of empirical exposure replicates, and a random dose-response parameter was selected from the series of bootstrapped dose-response parameters. Summary descriptive statistics for the Monte Carlo projection of estimated risk of EVD illness were derived. Risk magnitude was presented as an interval estimate using box plots to illustrate the confidence region and degree of precision with which the risk had been estimated based on the uncertainty and variability of the input quantities and the assumptions made in the QMRA.

**Results and Discussion**

The magnitude, variability, and uncertainty of the potential risk to collection system workers in the sewer line serving a hospital treating an Ebola patient was determined by integrating the results of the exposure and dose-response assessments.

**Exposure Assessment.** Values and distributions for the exposure assessment input parameters are summarized in Table 2. Parameter details and associated assumptions are discussed in the subsections that follow.

**Patient Excreta and Secreta Production to Sewer.** Patient excreta and secreta includes diarrhea, stool, vomit, urine, sweat, saliva, mucus, and tears (blood and serum are excluded). The daily volume of infectious liquid waste produced by EBOV patients fluctuates over the duration of the hospitalization period. Hospitalization periods for Ebola virus may last as long as 40 days depending on when the patient's bodily fluids cease to test positive for virus (Kreuels et al., 2014; Lyon et al., 2014; Wolf et al., 2014). This assessment assumes that all excreta and secreta produced by a patient is disposed of in the hospital sanitary sewer and that liquid wastes are not disinfected prior to disposal, which is the current CDC and WHO recommendation (CDC, 2014a, 2014b; WHO, August 2014).

The range of excreta and secreta volumes documented in the literature was 2 to 10 L/d (Bishop 2014; Chertow et al., 2014; Ker et al., 2015; Kreuels et al., 2014; Lowe et al., 2014; Lyon et al., 2014; Ribner, 2014; Roberts and Perner, 2014). Daily waste production reported for a single EBOV patient over 18 days (Kreuels et al., 2014) was fit using Crystal Ball and the recommended distribution was Logistic (\( \text{Mean} = 5.89; \text{Scale} = 1.04l \)).

**Excreta and Secreta Concentrations of EBOV.** Ebola virus concentration in patient excreta and secreta fluctuates over the duration of illness. Patient excreta has been shown to contain RNA copies beyond Day 40 and contain viable virus until Day 26 of illness (Kreuels et al., 2014).

Inhalation exposure in the sewer collection system was estimated in terms of both viral RNA copies and PFUs. At the time of writing, data for EBOV concentration in human excreta and secreta was only available in terms of RNA copies. The precise conversion from RNA copies to PFUs for EBOV is unknown, but a factor of \( 3.4\log_{10} \) RNA copies per PFU was applied based on findings for the *Ebolavirus Sudan strain* (Towner et al., 2004), implying that \( 3.4\log_{10} \) RNA copies per PFU are required to pose the same infection hazard as a PFU. This conversion factor was substantiated for EBOV by comparing excreta and secreta RNA copy concentrations from humans (Kreuels et al., 2014; Wolf et al., 2014), NHPs (Alimonti et al., 2014), and pigs (Weingartl et al., 2012) to PFU and TCID50 concentrations from NHPs (Prescott, 2015) and pigs (Kobinger et al., 2011).

Human excreta and secreta viremia data were only available for sweat, urine, and stool. The range of EBOV concentrations documented in the literature for these three media was \( 2.8 - 7.2\log_{10} \) viral RNA copies/mL excreta. Viral concentrations in other excreta and secreta media (diarrhea, vomit, saliva, mucus, and tears) were assumed to be represented by this range.

It was assumed that all patient excreta and secreta was mixed uniformly prior to disposal. Viremia data for sweat, urine, and stool over duration of illness (Kreuels et al., 2014, Wolf et al., 2014) was pooled and fit using Crystal Ball and the recommended distribution was Logistic (\( \text{Mean} = 4.38\log_{10}; \text{Scale} = 0.61\log_{10} \)).

**Hospital Daily Outflow (for Internal Dilution Rate in Hospital).** The volume of daily liquid outflows from four hospitals with Ebola treatment centers was established through conversations with utilities. It was assumed that all patient excreta and secreta mixed evenly with the hospital wastewater prior to discharge to the municipal sewer. Hospital wastewater discharge ranged from \( 3.63 \times 10^7 \) to \( 1.33 \times 10^9 \) L/d (Metropolitan Water Reclamation District of Greater Chicago, personal communication, April 2, 2015)

Because of the small amount of data, a distribution could not be fit using Crystal Ball, so a Lognormal distribution was assumed with parameters derived from the data provided by utilities (\( \text{Mean} = 8.27 \times 10^5; \text{Std. Dev.} = 4.06 \times 10^3; 2.5\% = 2.99 \times 10^3; 97.5\% = 1.85 \times 10^6 \)).

**Interceptor Daily Flow (for Dilution from Hospital Discharge to Sewer to Point of Worker Exposure).** The volume of daily flow through four municipal sewer interceptors that receive effluent from hospitals with Ebola treatment centers was also established through conversations with utilities. It was assumed that all hospital effluent mixed evenly with the wastewater in the municipal interceptor.Interceptor flows ranged from \( 1.82 \times 10^7 \) to \( 3.29 \times 10^8 \) L/d (Metropolitan Water Reclamation District of Greater Chicago, personal communication, April 2, 2015).

Because of the small amount of data, a distribution could not be fit to the data using Crystal Ball, so a Lognormal distribution was assumed with parameters derived from the data provided by
utilities (Mean = 1.61 × 10^8, Std. Dev. = 1.50 × 10^8; 2.5% = 2.50 × 10^7; 97.5% = 5.33 × 10^8).

Ebolavirus Die-Off Rate. Ebolavirus stability in water and air environments were investigated to determine whether the virus would undergo significant inactivation during transport from the patient to the sewer worker. Ebolavirus die-off kinetics in untreated wastewater are currently unknown, with studies in progress. At the time of writing, EBOV stability in water had only been examined for diethylpyrocarbonate-treated (DEPC) water. Thus, existing die-off data from DEPC water was assumed to be the best available representation of EBOV stability in wastewater.

Ebolavirus is shown to undergo a 1 log_{10} reduction every 1.8 days in DEPC-treated water at 21 °C (Fischer et al., 2015). This value was converted to a 6.43 × 10^{-8} log_{10} reduction per second and applied as a point value to the calculation in eq 1. The water temperature from the point of patient excretion to the point of sewer worker exposure was assumed to maintain a constant 21 °C. In a similar persistence study published at the time of writing, EBOV was shown to undergo a 1 log_{10} reduction every 2.1 days in treated wastewater, which does not materially change the conclusions presented here (Bibby et al., 2015).

Once aerosolized in the municipal interceptor, EBOV is expected to inactivate at a rate of 2.79 to 3.43% per second in air (Piercy et al., 2010; Smither et al., 2011). For this analysis, viral die-off during travel from the interceptor wastewater to the worker was assumed to be negligible. The short air travel time and the relative humidity and UV light conditions of the sewer were assumed to be supportive of viral particle persistence until inhalation.

Sewer Travel Time from Patient to Point of Worker Exposure. The travel time of the infectious waste from the patient source to the point of worker exposure was assumed to be negligible relative to the EBOV die-off rate in water. Pipe travel time from a hospital to a water resource recovery facility (WRRF) can be less than 1 hour depending on system dynamics (Ort et al., 2010; Weissbrodt et al., 2009), so travel time to the municipal interceptor is assumed to be on the order of minutes. Infectious EBOV waste is not expected to have undergone significant decay in this time. A point value of zero seconds was therefore assumed for the sewer travel time.

Partition Coefficient (Ratio of Aerosol Concentration of EBOV to Liquid Concentration). The concentration of EBOV RNA copies in the sewer headspace was determined using an air/water partition coefficient that relates the concentration of aerosolized microorganisms in sewer air to the concentration of microorganisms in sewer wastewater. This aerosolization ratio had not been documented for EBOV, but data was available in the literature to calculate the aerosolization efficiency of mesophilic heterotrophic bacteria in the sewer.

The physical process of producing airborne particles in the sewer is consistent across suspended and colloidal particles, but particles with greater hydrophobicity are expected to aerosolize more easily due to higher surface aggregation. For this analysis, the hydrophobicities of mesophilic heterotrophic bacteria and EBOV viral copies were assumed to be the same and the partition coefficient determined for mesophilic heterotrophic bacteria was applied to EBOV RNA copies.

Ranges for the partition coefficient for mesophilic heterotrophic bacteria were calculated from data in the literature where air samples and water samples were taken in the same location within the sewer collection system. In cases where only air samples were collected, the concentration of mesophilic heterotrophic bacteria in the wastewater was assumed to be in the range of 10^{10} to 10^{12} CFU/m^3 (Hung et al., 2010). Data was selected from locations within the sewer collection system that were expected to have a similar degree of turbulence as the point of worker exposure (duct, inlet to a duct, relief chamber, and WRRF intake screen).

Aerosolization ratios ranged from (−11.46 to −5.88) log_{10} (C_{\text{headspace}} / C_{\text{wastewater}}) (Dutkiewicz, 2003; Haas et al., 2002; Haas et al., 2010; Karra and Katsivela, 2007; Korzeniewska et al., 2009; Korzeniewska, 2011; Medema et al., 2004). This range encapsulates the −8 log_{10} partition coefficient used in previous QMRAs (Stellacci et al., 2010). Partition coefficient data was fit in Crystal Ball and a Beta distribution was recommended (Min = −11.46 log_{10}, Max = −5.88 log_{10}, Alpha = 2.3281 log_{10}, Beta = 1.96512 log_{10}).

Respirable Fraction (Fraction of Aerosols Generated That Are Respirable). To pose an infection risk via the respiratory pathway, aerosolized droplets containing microorganisms must exist in the headspace air within the size range capable of reaching the alveolar region of a sewer worker’s lungs. For this analysis, respirable droplets were assumed to have an aerodynamic diameter less than or equal to 3 μm (Dutkiewicz, 2003). Particles larger than 3 μm that get inhaled were assumed to be sequestered in the worker’s upper respiratory tract (Bray, 2003; Peters et al., 1996).

The fraction of droplets in the sewage collection system that are considered respirable varies depending on the degree of turbulence in the sampling location. Only one study collected respirable fraction data in sewer locations that simulate the environment at the point of worker exposure. These respirable fraction data from pump stations and sewer duct inlets ranged from 0.28 to 1.00 (Dutkiewicz, 2003). Because of the small amount of data, a distribution could not be fit to the data using Crystal Ball, so a Uniform distribution was assumed with parameters derived from the data (Min = 0.28, Max = 1.00).

Worker Inhalation Rate. The inhalation rate of the exposed sewer worker is variable depending on activity level and age. Short-term exposure values for inhalation from the U.S. Environmental Protection Agency (U.S. EPA) Exposure Factors Handbook were used, and it was assumed that the worker’s activity level at the point of exposure would be “high intensity” and that the worker would be 21 to 61 years old.

The range of inhalation rate data was assumed to follow a Normal distribution. Using the available mean and 95th percentile data, the inhalation rate for a given sewer worker was determined to have a mean of 51.0 L/min with a standard deviation of 14.9 L/min (U.S. EPA, 2011).

Ebolavirus Removal Fraction by Worker PPE. The CDC recommends that sewer collection workers wear proper PPE.
when handling untreated sewage that may contain EBOV. To protect from aerosols, it is recommended that workers use a National Institute for Occupational Safety and Health (NIOSH)-approved N-95 respirator that covers the nose and mouth (CDC, 2014b). N-95 respirators are engineered to filter at least 95% of particles that would be inhaled.

The removal fraction, however, varies depending on the degree of fit of the mask and the level of user compliance. N-95 respirators with a high fit factor provide a close face seal that prevents leakage. Fit testing is necessary to achieve the highest level of protection (Rengasamy et al., 2004). The U.S. Occupational Safety and Health Administration (OSHA) requires individual fit testing and the absence of interfering facial hair when using N-95 respirators (CDC: NPPTL, 2012). User compliance has also been documented as a risk because users may not tolerate discomfort or nasal airflow restriction for the entire exposure period (Gupta, 2011; Lee and Wang 2011). Workers may choose to wear a surgical mask, which has a much lower filtration efficiency and poorer face seal (Bazzy et al., 2006a; Gammatoni and Nucci, 1997; Gupta 2011; Oberg and Brosseau 2008; Rengasamy et al., 2004; Wen, Yu et al., 2013).

To encompass all scenarios of PPE usage, exposure was assessed for two situations: (1) the sewer worker fully complies with CDC guidance to wear a NIOSH-approved N-95 respirator and the respirator is properly fitted and worn at all times during handling of untreated sewage, and (2) the sewer worker fully ignores CDC guidance and no face protection is used.

Assuming the N-95 respirator performs with the same removal efficiency at the worker inhalation rate as it does when factory tested at a flow rate of 85 L/min, the fraction of particles removed in the first scenario ranges from 0.95 to 0.99 (Bazzy et al., 2006a; Balazy et al., 2006b; Gupta, 2011; Rengasamy et al., 2004; Wen et al., 2013). A distribution could not be fitted to the data using Crystal Ball, so a Uniform distribution was assumed with parameters derived from the data (Min = 0.95; Max = 0.99). The fraction of particles removed in the second scenario is zero.

**Time Spent by a Sewer Worker in the Proximity.** The point of worker exposure is taken to be the point in the municipal collection line immediately downstream of where the hospital sanitary discharge line intersects with the municipal interceptor. The amount of time that a sewer worker spends in this proximity depends on the activity the worker is performing. Common maintenance activities such as blockage removal, sampling, de-ragging, or jet operation can range from 0.5 to 4 hours in duration (Sanitation Districts of Los Angeles County, personal communication, April 3, 2015; Metropolitan Water Reclamation District of Greater Chicago, personal communication, April 2, 2015).

A distribution could not be fitted to the data using Crystal Ball, so a Uniform distribution was assumed with parameters derived from the data (Min = 0.95; Max = 4.0)

**Dose of Respirable EBOV Inhaled and Retained in Lungs per Exposure.** The exposure parameters were input into eq 1 in Crystal Ball to determine the dose of respirable EBOV inhaled and retained in a sewer worker’s lungs per exposure. Inhalation doses are presented in Table 3 in terms of RNA Copies and PFUs and according to degree of compliance with CDC guidance to wear a properly fitted, NIOSH-approved N-95 respirator.

When converting from units of RNA copies to PFUs, a Uniform distribution was assumed for the conversion factor of 3–4log_{10} RNA copies per PFU described previously (Min = 10^{3}; Max = 10^{4}). Thus, the inhaled dose in Scenario 2: PPE_PFU and Scenario 4: NoPPE_PFU represents the inhaled dose in Scenario 1: PPE_Gene and Scenario 3: NoPPE_Gene with the conversion distribution applied, respectively.

The results of the Monte Carlo analyses (10,000 trials) from each of the four scenarios (Table 3) illustrate that the dose of respirable EBOV inhaled by a sewer worker is lower when PPE recommendations are heeded.

A graphical output of the Monte Carlo results is illustrated in Figure 1 for Scenario 1: PPE_Gene. The probability-versus-dose histogram shows the frequency with which an exposure dose estimate appears across the 10,000 trials. For example, of the 10,000 dose estimates for Scenario 1: PPE_Gene, the probability that a dose estimate will be in the range of 10^{-7} to 10^{-6} RNA copies is approximately 22%. The cumulative percentage line shows the likelihood that a dose estimate is less than a given value. For Scenario 1: PPE_Gene, the graph shows a 95% likelihood that the dose of respirable EBOV RNA copies inhaled and retained by a worker that is fully compliant with PPE recommendations will be fewer than 3.65261log_{10} RNA copies (2.23 × 10^{-4} RNA copies).

A sensitivity analysis is shown in Figure 2 for Scenario 1: PPE_Gene. The directional bar graph reveals that the air/water partition coefficient (P_{AC}) and the excreta and secreta concentrations of EBOV (C_{AC}) contribute most to variability in dose outcome, with 54.0% and 37.4% of the output variability being attributed to these two inputs, respectively. This result suggests that focusing further data gathering efforts to reduce uncertainty for these two input parameters will have the greatest impact on improving the precision of the forecasted exposure dose.

The 10,000 dose estimates from the Monte Carlo simulations for each of the four scenarios were extracted and combined with

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### Table 3—Dose of respirable EBOV inhaled and retained in lungs per exposure (10 000 trials).

<table>
<thead>
<tr>
<th>Scenario ID</th>
<th>Scenario</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: PPE_Gene</td>
<td>Worker is fully compliant with PPE recommendations</td>
<td>95% probability that worker inhales fewer than 2.23 × 10^{-4} RNA Copies</td>
</tr>
<tr>
<td>2: PPE_PFU</td>
<td>Worker is noncompliant with PPE recommendations</td>
<td>95% probability that worker inhales fewer than 9.34 × 10^{-4} PFUs</td>
</tr>
<tr>
<td>3: NoPPE_Gene</td>
<td>Worker is noncompliant with PPE recommendations</td>
<td>95% probability that worker inhales fewer than 6.96 × 10^{-3} RNA Copies</td>
</tr>
<tr>
<td>4: NoPPE_PFU</td>
<td>Worker is noncompliant with PPE recommendations</td>
<td>95% probability that worker inhales fewer than 3.25 × 10^{-6} PFUs</td>
</tr>
</tbody>
</table>
the results of the dose-response assessment to establish the risk characterization.

Dose-Response Assessment. The best fit dose-response curve (exponential) is shown plotted against the experimental NHP dose-response data in Figure 3, with 95 and 99% confidence regions determined by bootstrapping (Mitchell et al., in preparation). Note that while the health outcome in the animal studies was death, the health outcome highlighted here is EVD illness. To correlate the two health outcomes, it was assumed that because animals do not receive medical intervention during testing, any animal that became ill with EVD died. This assumption justifies using NHP mortality studies as models for illness without modification.

The best fit parameter value and minimized deviance for the exponential model are listed in Table 4. The maximum likelihood estimate of the exponential dose-response parameter is \( k = 0.07577 \) and the goodness-of-fit statistic (minimized deviance) is 3.233, which is sufficient to deem the fit statistically acceptable.

The dose-response curve and statistics illustrate that EBOV is highly virulent, as only a small number of PFUs are required to reach the alveoli in order to survive host defenses, initiate infection, and cause EVD illness. For example, inhaling a dose of 1.4 PFUs corresponds to a likelihood of EVD illness of approximately 0.10 (10%). Small incremental increases in dose correspond to a steep increase in the likelihood of EVD illness, with 9.1480 PFUs (N50) and 30 PFUs corresponding to 0.50 (50%) and approximately 0.90 (90%) probabilities of EVD illness, respectively.

Bootstraping established the 95 and 99% confidence regions for the dose-response curve and provided 500 dose-response parameters (\( k \)) to be used in the risk characterization. By determining the best dose-response function to represent the relationship between EBOV inhalation dose and probability of EVD illness, the overall risk profile that a sewer worker faces when operating downstream from a hospital treating Ebola patients can be established.

Risk Characterization. The set of 10,000 exposure dose replicates for each of the four scenarios and the set of 500 dose-response parameters (\( k \)) were combined using eq 2 for the exponential dose-response distribution.

The exposure dose sets from Scenario 2: PPE_PFU and Scenario 4: NoPPE_PFU were input directly into the exponential model because the dose units matched those of the empirical NHP dose-response data used to derive the dose-response parameter (PFUs). The exposure dose sets from Scenario 1: PPE_Gene and Scenario 3: NoPPE_Gene did not have the

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**Figure 1**—Probability of inhaled dose (RNA copies) in Scenario 1: PPE_Gene (10 000 trials).

**Figure 2**—Sensitivity analysis from Monte Carlo simulation for Scenario 1: PPE_Gene.

**Figure 3**—Best-fit dose-response curve (exponential).
conversion of $3-4\log_{10}$ RNA copies per PFU applied, however, and could not be input directly into the exponential model without conversion to PFUs. For investigational purposes, these authors chose to examine what the risk of EVD illness would be if the conversion from RNA copies to PFUs was one-to-one (i.e., if the ability of an RNA copy to induce EVD illness was equivalent to that of a PFU). Such a conversion would represent the extreme upper bound of the infectivity of an RNA copy. Thus, a conversion factor of one was applied to the estimated dose distributions from Scenario 1: PPE_Gene and Scenario 3: NoPPE_Gene and the distributions were input into eq 2.

The summary statistics for the estimates of $\log_{10}$ risk of EVD illness computed by Monte Carlo simulation (1000 trials) are shown in Table 5 in order of increasing risk. In the case of Scenario 2: PPE_PFU, a number of the trials produced risk less than machine accuracy resulting in the minimum and mean being indeterminate. In the other three scenarios, the confidence region of the risk distribution was broad (max $\gg$ min); however, the skew was small (mean $\approx$ median). Of the four alternatives, Scenario 2: PPE_PFU exhibited the least risk with a median risk of EVD illness of $10^{-11}$ and Scenario 3: NoPPE_Gene showed the greatest risk with a median risk of approximately $10^{-5.77}$.

The resultant $\log_{10}$ risks of EVD illness for all four scenarios are represented in the box and whisker plot shown in Figure 4. The center line of each box signifies the median risk, and the lower and upper extents of the boxes denote the first and third quartiles. The upper and lower "whiskers" are at 1.5 times the interquartile range (distance between the first and third quartile) away from the median. Individual points are plotted at more extreme values. The plot shows the overall risk profiles of Scenario 2: PPE_PFU and Scenario 4: NoPPE_PFU are lower than those of Scenario 1: PPE_Gene and Scenario 3: NoPPE_Gene, illustrating that applying a $3-4\log_{10}$ RNA copies to PFU conversion factor results in a lower overall risk profile than using a one-to-one conversion factor regardless of PPE compliance.

The results show that calculating the risk of EVD illness using a conversion factor of one to relate RNA copies to PFUs results in a much higher risk than using the $3-4\log_{10}$ conversion factor described in the literature. Additionally, effective use of PPE is shown to decrease the worker’s overall risk of EVD illness. Consequently, the median risk in Scenario 3: NoPPE_Gene ($10^{-5.77}$) is quite high, at approximately $10^5$ times the median risk of Scenario 2: PPE_PFU ($10^{-11}$).

The substantial contrast between the least favorable and most favorable conditions illustrates that a significant unknown is the actual conversion from EBOV RNA copies to PFUs in the environment in which exposure occurs. The published conversion of $3-4\log_{10}$ has only been examined in one study for the Ebolavirus Sudan strain, and represents the ratio between RNA copies and culturable virus. It does not account for viable non-culturable virus, that is, the viral particles that may not form a plaque in laboratory cell media but may cause infection in a host. The ratio between RNA copies and infectious particles needs to be better assessed (particularly after some transport in the environment).

When interpreting the indications of these risk estimates, one must consider that the following conservative assumptions were made:

- The point of worker exposure was chosen to be the closest point to the hospital sanitary discharge line. Ebola virus viral particles would have experienced the least opportunity for dilution or die-off at this point.
- The EBOV die-off rate in untreated wastewater was assumed to be the same as DEPC–treated water. In persistence studies, enveloped viruses like EBOV have been shown to die off in sewage much faster than in treated water due to damage to the lipid envelope by physical, chemical, and biological agents in the environment (Casanova et al., 2009; WHO, March 2015). Additionally, recent results from a persistence study in treated wastewater suggest that the upper bound for $1\log_{10}$ inactivation of EBOV in wastewater is 2.1 days (Bibby et al., 2015), implying that inactivation in untreated sewage would be faster. However, if the transit time within the hospital until mixing with the bulk interceptor sewage is sufficiently rapid, decay will remain negligible.
- The sewer travel time within the hospital was assumed to be negligible. In reality, a small degree of virus inactivation would be expected during transport. Additionally, for hospitals with longer sewer travel times, greater inactivation during transport would be expected.

### Table 4—Best-fit dose-response parameter value and deviance (exponential).

<table>
<thead>
<tr>
<th>Minimized Deviance</th>
<th>$k$</th>
<th>$N_{0}$</th>
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<td>3.233</td>
<td>0.07577</td>
<td>9.1480</td>
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</table>

### Table 5—Summary of descriptive statistics of $\log_{10}$ risk (1000 trials) per exposure.

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<tr>
<th>Minimum</th>
<th>1st Quartile</th>
<th>Median</th>
<th>Mean</th>
<th>3rd Quartile</th>
<th>Maximum</th>
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</table>
Figure 4—Box and whisker plot of log10 risk (1000 trials).

Additional assumptions made in this analysis require further investigation to improve the precision of the risk profile:

- All patient excreta and secreta were assumed to mix evenly with the hospital wastewater prior to discharge to the municipal sewer. It is more likely that liquid infectious waste would enter the wastewater stream in concentrated “pulses” rather than a continuous stream, resulting in episodically higher exposure doses.
- Hospitals that accept Ebola patients in smaller municipalities or other countries may have lower dilution ratios than those in Chicago and Los Angeles, resulting in higher exposure doses in the sewer.
- The hydrophobicities of mesophilic heterotrophic bacteria and EBOV viral particles were assumed to be the same. If EBOV was found to be more hydrophobic, its transfer to ambient air would be expected to be greater.

Conclusion

The results of this QMRA suggest that the potential risk that sewer workers face when operating in a wastewater collection system downstream from a hospital treating an Ebola patient warrants further attention. While an acceptable risk of EVD illness has not yet been defined, under the least favorable conditions in which PPE is not worn and EBOV RNA copies are deemed as virulent as PFUs (Scenario 3: NoPPE_Gene), the median potential risk of developing EVD illness from inhalation exposure to EBOV-contaminated aerosols in the sewer is approximately \(10^{-5.77}\) (with a first to third quartile range of \(10^{-7.06}\) to \(10^{-4.65}\)), a value higher than many risk managers may be willing to accept. Thus, current WHO and CDC guidance for EBOV liquid waste disposal—to dispose in the sanitary sewer with disinfectant prior to discharge to the sewer, which, if effective, would accelerate the inactivation of viral particles and reduce inhalation exposure downstream.

The uncertainties of the four risk projections presented in this study can be reduced by focusing future data-gathering efforts on the three input parameters that contributed most to the uncertainty in the risk characterization outcome: the ratio between EBOV RNA copies and PFUs, excreta and secreta concentrations of EBOV (\(C_{ES}\)), and the air/water partition coefficient \(P_{x}\) specific to EBOV. Establishing narrower confidence intervals for these parameter distributions will help determine which of the four scenarios highlighted in this study most accurately represents risk of developing EVD illness from inhalation exposure in the sewer.

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